FILE 'BIOSIS, HCAPLUS' ENTERED AT 15:13:03 ON 06 FEB 2003

L1	41670	S FLUORESCEIN
L2	524626	S EMIT OR EMISSION
L3	14420	S CYANINE
L4	1237	S TEXAS RED
L5	333	S L1 (7A) L2
L6	206777	S WAVELENGTH
L7	9031	S L2 (2A) L6
L8	18	S L7 (5A) L1
L9	15	DUP REM L8 (3 DUPLICATES REMOVED)
L10	6	S L7 (5A) L3
L11	4	DUP REM L10 (2 DUPLICATES REMOVED)
L12	2	S L7 (5A) L4

L13 ANSWER 24 OF 27 HCAPLUS COPYRIGHT 2003 ACS

AB A rapid and sensitive homogeneous assay method has been developed for the detn. of subtilisin. The method employs a protein **substrate** **labeled** with two fluorescent dyes with fluorescence energy transfer (FET) characteristics. The doubly-**labeled** **substrate** was prepd. by chem. coupling bovine serum albumin with lucifer yellow and **rhodamine** dyes. The fluorescence emission from the lucifer labels was initially quenched due to the FET to the adjacent **rhodamine** labels. However, upon the addn. of subtilisin into the **labeled** **substrate** soln., increased fluorescence was obsd. as the enzyme hydrolyzed the substrate and reduced the FET effect. The rate of increase in fluorescence due to substrate hydrolysis was used to calibrate the subtilisin assay. It was linear over the range 0-150 ng of the enzyme (r2=0.985). The assay was fast with a time of 30 s to exceed the limit of detection (LOD) signal for 60 ng of subtilisin in 600 .mu.l. In this vol., the LOD for the enzyme was 4.2 ng (99% confidence).

AN 1996:616420 HCAPLUS

DN 125:268804

TI A rapid homogeneous fluorescence assay for subtilisin

AU Tang, Lian X.; Rowell, Frederick J.; Cumming, Robert H.

CS Sch. Health Sci., Univ. Sunderland, Sunderland, SR1 3SD, UK

SO Analytical Letters (1996), 29(12), 2085-2095
CODEN: ANALBP; ISSN: 0003-2719

PB Dekker

DT Journal

LA English

L13 ANSWER 10 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AB The kinetics of PaeR7 endonuclease-catalyzed cleavage reactions of fluorophor-**labeled** oligonucleotide **substrates** have been examined using fluorescence resonance energy transfer (FRET). A series of duplex substrates were synthesized with an internal CTCGAG PaeR7 recognition site and donor (fluorescein) and acceptor (**rhodamine**) dyes conjugated to the opposing 5' termini. The time-dependent increase in donor fluorescence resulting from restriction cleavage of these substrates was continuously monitored and the initial rate data was fitted to the Michaelis-Menten equation. The steady state kinetic parameters for these substrates were in agreement with the rate constants obtained from a gel electrophoresis-based fixed time point assay using radiolabeled substrates. The FRET method provides a rapid continuous assay as well as high sensitivity and reproducibility. These features should make the technique useful for the study of DNA-cleaving enzymes.

AN 1994:448298 BIOSIS

DN PREV199497461298

TI Real time kinetics of restriction endonuclease cleavage monitored by fluorescence resonance energy transfer.

AU Ghosh, Soumitra S.; Eis, Peggy S.; Blumeyer, Kirsten; Fearon, Kim; Millar, David P. (1)

CS (1) Cripps Res. Inst., La Jolla, CA 92037 USA

SO Nucleic Acids Research, (1994) Vol. 22, No. 15, pp. 3155-3159. ISSN: 0305-1048.

DT Article

LA English

FILE 'BIOSIS, HCAPLUS' ENTERED AT 15:19:04 ON 06 FEB 2003

L1	1062661	S	SUBSTRATE
L2	76357	S	FLUORESCEIN OR RHODAMINE OR CYANINE
L3	1237	S	TEXAS RED
L4	26571	S	?RHODAMINE
L5	49830	S	?FLUORESCEIN
L6	14420	S	CYANINE
L7	7	S	L1 (7A) L3
L8	6	DUP REM	L7 (1 DUPLICATE REMOVED)
L9	485	S	L1 (7A) L4
L10	9817	S	LABEL? (7A) L1
L11	60	S	L10 (P) L4
L12	41	DUP REM	L11 (19 DUPLICATES REMOVED)
L13	27	S	L12 NOT PY>1999
L14	216	S	L10 (P) L5
L15	204	S	L14 NOT L11
L16	203	S	L15 NOT L7
L17	154	S	L16 NOT PY>1999
L18	101	DUP REM	L17 (53 DUPLICATES REMOVED)
L19	717851	S	DUAL OR DOUBLE OR HOMO
L20	5	S	L19 (P) L14
L21	4	DUP REM	L20 (1 DUPLICATE REMOVED)
L22	9	S	L10 (P) L6
L23	7	DUP REM	L22 (2 DUPLICATES REMOVED)
L24	5	S	L23 NOT L11

L Number	Hits	Search Text	DB	Time stamp
1	1520630	substrate	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/06 15:32
2	1110514	dual or double or homo	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/06 15:32
3	159674	label	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/06 15:32
4	221041	fluoresc$5	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/06 15:32
5	15137	(dual or double or homo) near5 label	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/06 15:32
6	373	substrate same ((dual or double or homo) near5 label)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/06 15:33
7	0	(substrate same ((dual or double or homo) near5 label)) same fluoresc$5	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/06 15:33
8	15	(substrate same ((dual or double or homo) near5 label)) same dye	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/06 15:36